University of Utah
UNDERGRADUATE RESEARCH JOURNAL

PRIMARY T CELLS TRANSDUCED WITH INSULIN SPECIFIC TCRS HAVE FUNCTIONAL TCR SIGNALING FOLLOWING TETRAMER STIMULATION

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Type 1 diabetes (T1D) is an autoimmune form of diabetes which results from an adaptive immune response against islet self-antigens in the pancreas. T1D is an organ-specific autoimmune disease where T and B lymphocytes recognize islet antigens and subsequently destroy insulin producing beta cells. The body has multiple defense mechanisms to protect against autoimmunity, one of which are T regulatory cells (Tregs), a subset of CD4 T lymphocytes. Tregs have a variety of suppressive functions, such as the production of immunosuppressive cytokines, which are regulated through signaling by T cell receptors (TCRs)[1]. TCRs are protein complexes present on T cells that recognize antigens presented by major histocompatibility complexes (MHCs), and when activated propagate signals to the T cell. In T1D, these TCRs have the potential to respond to insulin antigens produced by beta cells, which are taken up by antigen presenting cells (APCs) and presented to CD4 T cells. CD4 T cells then activate macrophages, as well as other CD4 and CD8 cells, leading to the targeted destruction of beta cells. A hypothesized treatment for T1D involves improving trafficking of peripheral Tregs to the pancreas through modification of their TCR[2]. This approach requires investigation into TCR affinity and downstream signaling processes. In this project, two insulin specific TCR vectors 12-4.4m1 and 4-8, with low and high affinities respectively, were used to transduce CD4 T cells from NOD Nur77-GFP mice, and their TCR signaling strength and function were examined. We hypothesized that both the low and high affinity TCR vectors will show functional TCR signaling, however the lower affinity TCR 12-4.4m1 will have a lower level of TCR signaling compared to the higher affinity TCR 4-8. Based on Nur77-GFP expression, we demonstrate that TCR signaling is active in these transduced T cells. Additionally, we show a higher level of Nur77-GFP expression in 4-8 transduced T cells compared to 12-4.4m1, which confirms the hypothesis that 4-8 induces higher TCR signaling in transduced T cells than 12-4.4m1 following tetramer stimulation.

Figure 1: TCR Affinity Contributes to Differences in TCR Signaling in Transduced Primary CD4 T Cells

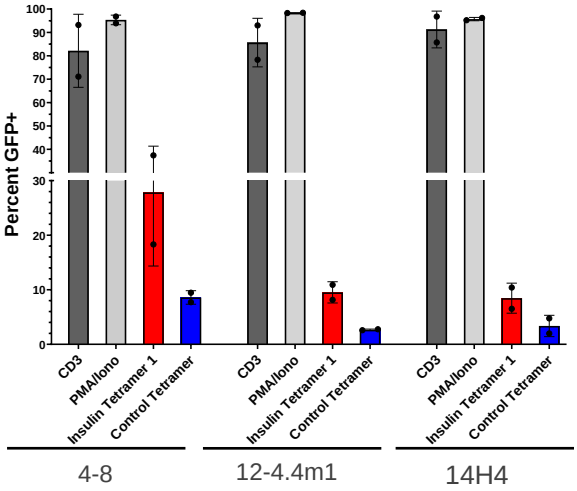
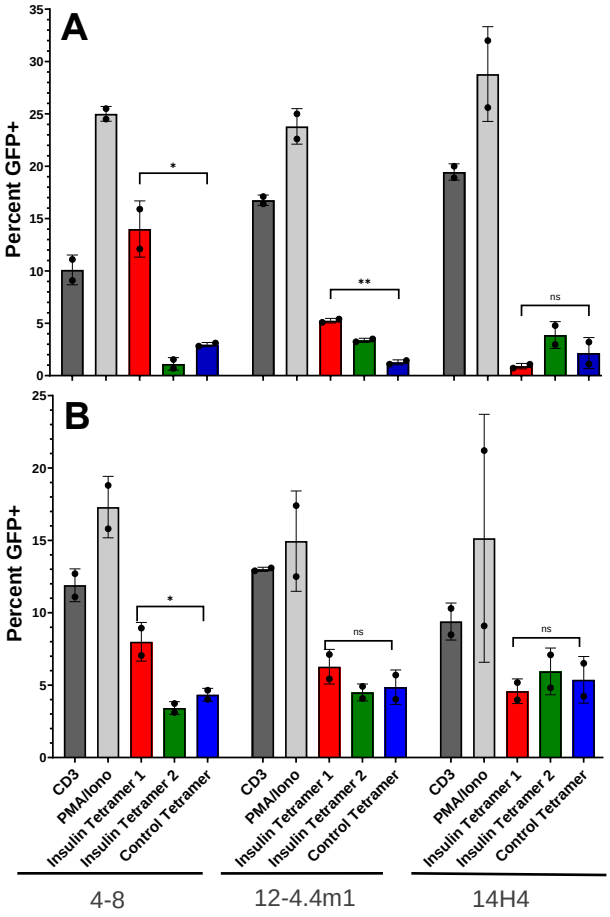


FIGURE 1 | *Nur77-GFP expression of transduced T cells 5 hours following stimulation, determined by flow cytometry data. PMA/Ionomycin and αCD3 are the positive controls and their high level of GFP show that the cells were properly stimulated. Results show that insulin tetramer 1 induces higher GFP expression in insulin specific TCRs compared to control TCR vector 14H4. Additionally, insulin tetramer 1 induces higher GFP expression in high affinity 4-8 TCR transduced T cells compared to low affinity 12-4.4m1 transduced T cells.*

Figure 2: Transduced Sorted CD4 T Cells Similarly Indicate the Role of TCR Affinity in Differing TCR Signaling

FIGURE 2 | *Nur77-GFP expression in sorted CD4+ T cells (T effectors (Teffs) and Tregs), 5 hours following stimulation, determined using flow cytometry data. (**A**) **Teff** cells stimulated under similar conditions as Figure 1. Statistically significant differences in GFP expression between insulin tetramer 1 and control tetramer in both 4-8 and 12-4.4m1 TCRs are observed. For 4-8 TCR, the insulin tetramer stimulation resulted in over double the amount of GFP expression compared to 12-4.4m1 TCR, indicating that high affinity 4-8 TCR has stronger TCR signaling. Unpaired T Test *p<0.05, **p<0.005 (**B**) **Treg** cells stimulated under similar conditions as Figure 1. Statistically significant differences in GFP expression observed between insulin tetramer 1 and control tetramer in 4-8 TCR only. 12-4.4m1 TCR data suggests a similar trend in difference in GFP expression between insulin tetramer 1 and control tetramer. 4-8 TCR demonstrates stronger TCR signaling than 12-4.4m1 TCR, indicated by its higher level of GFP expression. Unpaired T Test *p<0.05.*



References

[1] Bettini, M., Scavuzzo, M. A., Liu, B., Kolawole, E., Guo, L., Evavold, B. D., Borowiak, M., & Bettini, M. L. (2020, March 1). *A critical insulin tcr contact residue selects high-affinity and pathogenic insulin-specific t cells*. Diabetes. https://diabetes.diabetesjournals.org/content/69/3/392.

[2] Sprouse ML, Shevchenko I, Scavuzzo MA, Joseph F, Lee T, Blum S, Borowiak M, Bettini ML, Bettini M. Cutting Edge: Low-Affinity TCRs Support Regulatory T Cell Function in Autoimmunity. J Immunol. 2018 Feb 1;200(3):909-914. doi: 10.4049/jimmunol.1700156. Epub 2017 Dec 27. PMID: 29282307; PMCID: PMC5962277.